

2008

Application No.: 10/680,963  
Amendment Date: September 15,

Reply to Office Action of: 28 May 2008

### REMARKS/ARGUMENTS

Claims 76-96 are pending, Claims 1-12 have been withdrawn, and Claims 13-75 have been cancelled. Claims 97-99 are new.

Claims 1, 2, 3, 76, 81, and 86 have been amended to recite that the host cell has been genetically engineered to be diminished or depleted in the activity of an initiating  $\alpha$ -1,6-mannosyltransferase and to include at least an  $\alpha$ -1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity. Support for the amendments can be found throughout the specification, for example, generally in paragraphs [0129] and [0135]; engineering cells to have diminished an initiating  $\alpha$ -1,6-mannosyltransferase activity and to include at least an  $\alpha$ -1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity, paragraphs [0148]-[0161], [0162]-[0166] and the examples, e.g., Example 8.

Claims 76, 81, and 86 have been further amended to recite that the host cells comprise "a nucleic acid molecule encoding" the recited activity. Support for this amendment can be found throughout the specification, for example, in the examples (e.g., Example 8) and generally in paragraphs [0192] and [0206].

Claim 83 has been amended to recite that the host cell further includes a nucleic acid molecule encoding mannosidase II. Support for this amendment can be found for example in Example 14 or 15.

Claim 87 has been amended to recite that the host cell further includes a nucleic acid molecule encoding GnT II. Support for this amendment can be found for example in Example 11 or 15.

Claims 97-99 recite that the host cell is a methylotrophic yeast. Support for this amendment can be found in the fact that *Pichia* spp. such as *Pichia pastoris* are methylotrophic yeast. Other methylotrophic yeast include *Hansenula polymorpha*.

It is believed that the amendments to the claims have not introduced new matter into the application.

#### **I. Claims 76-96 have been rejected under 35 U.S.C. § 112, first paragraph.**

The rejection states that the claims fail "to comply with the written description requirement" because it is a "a new matter rejection." The rejection states that

amended claims 76, 81, and 86 recite "a yeast host cell which is diminished or depleted in the activity of an initiating  $\alpha$ 1,6-mannosyltransferase and includes an  $\alpha$ 1,2-mannosidase activity and a . . . " Applicants assert that the support for such

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amendment can be found in examples 19-21 as exemplified by strain YSH-1, YSH-44, YSH-57 and PBP38. A review of the application reveals that all these strains belong to *Pichia pastoris*. The fact of working example in the specification describes the specific generation of such a yeast host cell of one specie that applicant may now wish to extend a broadly covered by the scope of the new claims after the filing date of the as-filed application, does not provide any legal basis showing the applicant is possesses the specified subject matter as claimed in the new claims at the time the invention was made. Thus, this is a new matter rejection."

The rejection further states that while the

specification describes several strains of *P. pastoris* that are genetically modified or engineered to depleted with activity of  $\alpha$ 1,6-mannosyltransferase and include additional enzyme activities by introducing glycosylation enzymes that normally functions in human cells . . . , the specification does not describe any naturally occurring yeast host of other species or genetically modified yeast species. As such, since the original as-filed-specification does not support the newly added limitation, it constitutes new matter.

The claims have been amended to recite that the yeast host cells have been genetically engineered to be diminished or depleted in the activity of an initiating  $\alpha$ -1,6-mannosyltransferase and to include at least an  $\alpha$ -1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity. Genetically engineered yeast host cell having the above attributes are described in the specification and are exemplified in the examples. The examples use the methylotrophic yeast *Pichia pastoris* as a model organism to illustrate the construction of a genetically engineered that is deficient in initiating  $\alpha$ -1,6-mannosyltransferase and to include at least an  $\alpha$ -1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity. The examples further show modifying the host cell to include a GnT III activity and in further examples, modifying the host cell to include other glycosylation enzymes such as mannosidase II and GnT II and/or include other deletions such as rendering the host cell deficient in *alg3* activity. The genetically engineered host cells produced using *P. pastoris* as a model were capable of making glycoproteins that have bisected N-glycans. Thus, it is believed that the currently amended claims do not contain new matter and as such, satisfy 35 U.S.C. § 112, first paragraph.

While most steps in the process have been exemplified with the methylotrophic yeast *P. pastoris*, Example 9 teaches the production of a *K. lactis* yeast strain that is diminished or depleted in the activity of an initiating  $\alpha$ -1,6-mannosyltransferase. The example further shows

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that the N-glycans produced by the cell are a substrate for  $\alpha$ -1,2-mannosidase activity *in vitro* to produce Man<sub>5</sub>GlcNAc<sub>2</sub> N-glycans. This result shows that *K. lactis*, which is diminished or depleted in the activity of an initiating  $\alpha$ -1,6-mannosyltransferase, would be suitable for further genetic engineering as taught using *P. pastoris* as a model to produce a *K. lactis* host cell that includes at least an  $\alpha$ -1,2-mannosidase activity and a GnT I activity and further includes a GnT III activity.

To facilitate construction of genetically engineered yeast as currently claimed, the specification teaches a library method for constructing genetically engineered yeast cells that are capable of producing complex or hybrid N-glycans. The principle of the method is built upon the concept of putting the "right" enzyme in the "right" place. This is achieved by targeting the "right" enzyme to the "right" place via a heterologous targeting signal peptide to the ER or Golgi such that the "right" enzyme has optimal activity to process an N-glycan at a particular place in the pathway to produce an optimal amount of a particular N-glycan structure. This method was used successfully by the applicants to construct *P. pastoris* host cells that can make hybrid and complex N-glycans on a glycoprotein. This method can be used with any yeast species (or other organism for that matter) to produce host cells that make any variety of complex or hybrid N-glycans. Thus, the instant application teaches methods for making host cells diminished or depleted in the activity of an initiating  $\alpha$ -1,6-mannosyltransferase and to include at least an  $\alpha$ -1,2-mannosidase activity and a GnT I activity. These host cells can then be further modified to include one or more additional glycosylation enzymes such as GnT II or mannosidase II.

Thus, a person skilled in the art would appreciate that the methods taught in the specification may be used to genetically engineer any yeast species to produce the currently claimed host cells. For example, the *K. lactis* mutant of Example 9 can be transformed with nucleic acid molecules encoding an  $\alpha$ -1,2-mannosidase activity, a GnT I activity, and a GnT III activity. The applicants' library method would enable identification of host cells that optimally expressed each of the enzymatic activities. This host cell would be expected to produce GlcNAc<sub>2</sub>Man<sub>5</sub>GlcNAc<sub>2</sub> N-glycans in an *alg3*<sup>+</sup> host and GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> N-glycans in an *alg3*<sup>-</sup> host. The further transformation with a nucleic acid molecule encoding a mannosidase II would produce GlcNAc<sub>3</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> N-glycans in either host.

Therefore, in light of the above, it is believed that a person skilled in the art in light of the applicants' teachings in the specification would have understood the applicants to be in possession of the currently claimed host cells. Reconsideration of the rejection is requested.

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## II. Specification

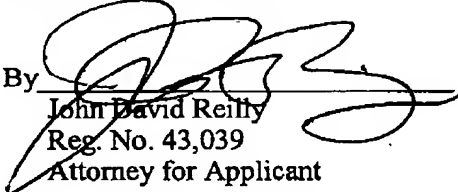
The amendment made to the specification in the response mailed on 27 Aug 2007 was objected to for disclosing new matter into the disclosure of the invention. The added matter alleged was the cancellation of various elements in the definition of lower eukaryotes. While respectfully disagreeing with the objection, the response of 28 February 2008 reintroduced the material that had been cancelled in the 27 Aug 2007 response. The intent of the 28 February 2008 response was to make clear that the definition of lower eukaryotes is to be as stated in the originally filed application.

### CONDITIONAL PETITION

Applicant hereby makes a Conditional Petition for any relief available to correct any defect in connection with this filing, or any defect remaining in this application after this filing. The Commissioner is authorized to charge deposit account 13-2755 for the petition fee and any other fee(s) required to effect this Conditional Petition.

Respectfully submitted,

By

  
John David Reilly  
Reg. No. 43,039  
Attorney for Applicant

MERCK & CO., INC.  
P.O. Box 2000  
Rahway, New Jersey 07065-0907  
(732) 594-6914

Date:

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